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ACCESSION NUMBER: 2001336287 MEDLINE
TITLE: Pyruvate/dichloroacetate supply during reperfusion accelerates recovery of cardiac energetics and improves mechanical function following cardioplegic arrest.
AUTHOR: Smolenski R T; Amrani M; Jayakumar J; Jagodzinski P; Gray C C; Goodwin A T; Sammut I A; Yacoub M H
SOURCE: EUROPEAN JOURNAL OF CARDIO-THORACIC SURGERY, (2001 Jun) 19 (6) 865-72.

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2.

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ACCESSION NUMBER: 2002121773 MEDLINE
TITLE: Energetic stimulation of the heart.
AUTHOR: Hermann H P
SOURCE: CARDIOVASCULAR DRUGS AND THERAPY, (2001 Sep) 15 (5) 405-11.

3.
ACCESSION NUMBER: 85239005 MEDLINE
TITLE: The effects of four different crystalloid bypass pump-priming fluids upon the metabolic response to cardiac operation.
AUTHOR: McKnight C K; Elliott M J; Pearson D T; Holden M P; Alberti K G
SOURCE: JOURNAL OF THORACIC AND CARDIOVASCULAR SURGERY, (1985 Jul) 90 (1) 97-111.

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Pyruvate/dichloroacetate supply during reperfusion accelerates recovery of cardiac energetics and improves mechanical function following cardioplegic arrest

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Abstract

Objectives: Cardioplegic arrest during cardiac surgery induces severe abnormalities of the pyruvate metabolism, which may affect functional recovery of the heart. We aimed to evaluate the effect of pyruvate and dichloroacetate administration during reperfusion on recovery of mechanical function and energy metabolism in the heart subjected to prolonged cardioplegic arrest. **Methods**: Four groups of rat hearts perfused in working mode were subjected to cardioplegic arrest (St. Thomas' No. 1), 4 h of ischaemia at 8°C and reperfusion with either Krebs buffer alone (C) or with 2.8 mM pyruvate (P), with 1 mM dichloroacetate (D), or with a combination of both (PD). Mechanical function was recorded before cardioplegic arrest and at the end of experiments. In groups C and PD, additional experiments were performed using ^{31}P nuclear magnetic resonance spectroscopy in non-working Langendorff mode to evaluate cardiac high-energy phosphate concentration changes throughout the experiment. **Results**: Improved recovery of cardiac output (% of the preischaemic value ± SEM, $n = 9$–12) was observed in all three treated groups (65.7 ± 4.3, 59.5 ± 5.2 and 59.5 ± 5.3% in PD, P and D, respectively) as compared with C (42.2 ± 4.6%; $P < 0.05$). Recovery of coronary flow was improved from 66.4 ± 3.8 in C to 94.9 ± 8.6% in PD ($P < 0.05$). The phosphocreatine recovery rate in the first minutes of reperfusion was increased from 9.9 ± 1.5 in C to 31.5 ± 4.3 μmol/min per g dry wt in PD ($P < 0.001$). No differences were observed in ATP or phosphocreatine concentrations at the end of experiment. **Conclusions**: The administration of pyruvate and dichloroacetate improves the recovery of mechanical function following hypothermic ischaemia. Accelerated restoration of the energy equilibrium in the initial phase of reperfusion may underlie the metabolic mechanism of this effect. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Pyruvate; Dichloroacetate; Heart transplantation; High-energy phosphates; Magnetic resonance spectroscopy; Cardioprotection

1. Introduction

Transient myocardial ischaemia induces a number of metabolic changes in the myocardium that could influence the recovery of the mechanical function. One of these changes is an altered metabolism of pyruvate, resulting from inhibition of the pyruvate dehydrogenase complex [1]. This, in turn, may affect the efficiency of energy production in the heart, both in the glycolytic pathway and in the tricarboxylic acid cycle, leading to a deterioration of mechanical function. We have previously demonstrated severe abnormalities of pyruvate metabolism in the heart under clinical conditions after heart transplantation [2]. This supports the view that amelioration of this problem may improve myocardial function under clinical conditions. This can be achieved through the administration of pyruvate or activation of the enzymes involved in its metabolism.

Dichloroacetate is a potent inhibitor of a protein kinase responsible for phosphorylation of the pyruvate dehydrogenase complex. This compound thus prevents transition of this enzyme from the active dephospho to the inactive phosphorylated form [3]. As a consequence, the pyruvate dehydrogenase complex remains in an active state, resulting in a shift of balance between glycolysis and glucose oxidation which facilitates metabolic and mechanical recovery after ischaemia [4,5]. The beneficial effects of dichloroacetate have been confirmed in experiments using isolated [illegible] experiment [infarction model]

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tions in patients with congestive heart failure [8]. Pyruvate supply during reperfusion following normothermic ischaemia was also found to improve heart performance [9]. A significant improvement in cardiac mechanical function was observed following intracoronary infusion of pyruvate in patients with heart failure [10].

The protective effects of dichloroacetate and pyruvate under conditions mimicking ischaemia during cardiac surgery have received little attention so far. To address this problem, we evaluated effect of pyruvate/dichloroacetate on cardiac mechanical function and the dynamics of metabolic changes using a perfused rat heart and nuclear magnetic resonance (NMR) spectroscopy in a protocol mimicking preservation of the heart for transplantation.

2. Materials and methods

2.1. Animals, heart collection and perfusion conditions

All animals received humane care in compliance with the 'Guide for the Care and Use of Laboratory Animals' published by the National Institutes of Health (NIH publication no. 85–23, revised 1985). Male Sprague–Dawley rats, weighing 300–350 g (Harlan-Olac, UK), were used in this study. The animals were anaesthetized with diethyl ether, and sodium heparin (1000 IU/kg) was then administered intravenously. Hearts were quickly excised and placed in cold Krebs–Henseleit buffer, consisting of 118.5 mM NaCl, 25 mM $NaHCO_3$, 1.2 mM $MgSO_4$, 4.8 mM KCl, 1.2 mM KH_2PO_4, 11 mM glucose, 2.8 mM lactate, 0.1 mM pyruvate and 1.4 mM Ca^{2+}, and were rapidly connected to the perfusion apparatus. Perfusion was carried out with the same Krebs–Henseleit buffer continuously gassed with 95% O_2/5% CO_2 at 37°C in both working mode and NMR experiments.

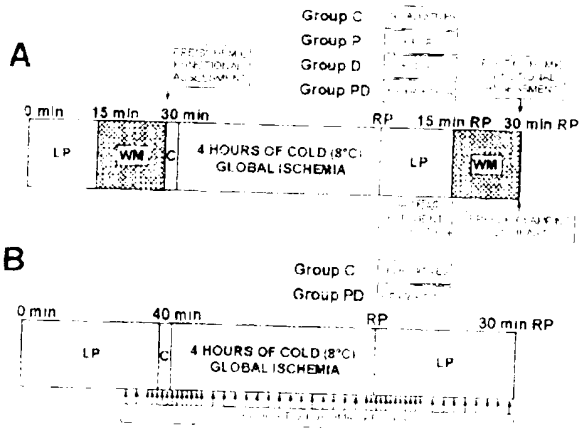
2.2. Perfusion conditions and functional assessment in working mode and Langendorff NMR experiments

The perfusion conditions during working mode experiments were similar to those previously described [11]. Briefly, the aorta and left atrium were cannulated and hearts were perfused with a preload pressure equivalent to 15 cm H_2O and afterload equivalent to 100 cm H_2O. The aortic flow was measured using a flow meter inserted into the aortic outflow line. Coronary flow was evaluated by the timed collection of the coronary effluent. The cardiac output was the sum of the aortic flow and coronary flow. Peak aortic pressure (PAP) readings were obtained from the pressure transducer located at the level of the heart and connected to the aortic cannula. The signal was also used to obtain the time derivative of the pressure changes. All recordings were performed using a Gould chart recorder. In Langendorff perfusion experiments for NMR, hearts were [illegible] in detail previously [12]. A fluid filled balloon was placed inside the left ventricle. The end-diastolic left ventricular pressure was maintained at the equivalent of 10 mmHg during perfusion and the balloon was off loaded during ischaemia. Hearts were not paced during working mode or NMR experiments.

2.3. Experimental protocol

2.3.1. Working mode

The experimental protocol is shown in Fig. 1A. In working heart model experiments, after an initial 15 min of Langendorff perfusion, the conditions were changed to working mode. At the end of 15 min of further perfusion, the baseline left ventricular function was evaluated by recording the aortic flow, coronary flow and PAP. Then, the hearts were arrested by the infusion of cold (8°C) St. Thomas' Hospital cardioplegic solution No. 1 (Martindale Pharmaceuticals, UK) at a constant pressure of 60 cm H_2O for 2 min. Finally, the hearts were immersed in cardioplegic solution and stored for 4 h at 8°C. At the end of the preservation period, the hearts were reperfused at 37°C in the Langendorff mode with Krebs–Henseleit buffer alone (C; $n = 12$), or with Krebs buffer with 2.9 mM pyruvate (P; $n = 9$), Krebs buffer with 1 mM dichloroacetate (D; $n = 9$), or Krebs buffer with 1 mM dichloroacetate and 2.9 mM pyruvate (PD; $n = 10$) for the first 15 min of reperfusion. Coronary effluent was collected throughout this 15 min of reperfusion; the volume was recorded and small aliquots were taken after mixing for determination of purine release from the heart. Another aliquot was taken for determination of troponin I release. After 15 min of Langendorff mode, the perfusion was switched to working mode and after 15 min, the mechanical function was evaluated. At the end of the perfusion protocol, hearts were freeze-clamped for analysis of nucleotide contents. All hearts which entered the experiment maintained stable haemodynamic function during the preischaemic phase and none of



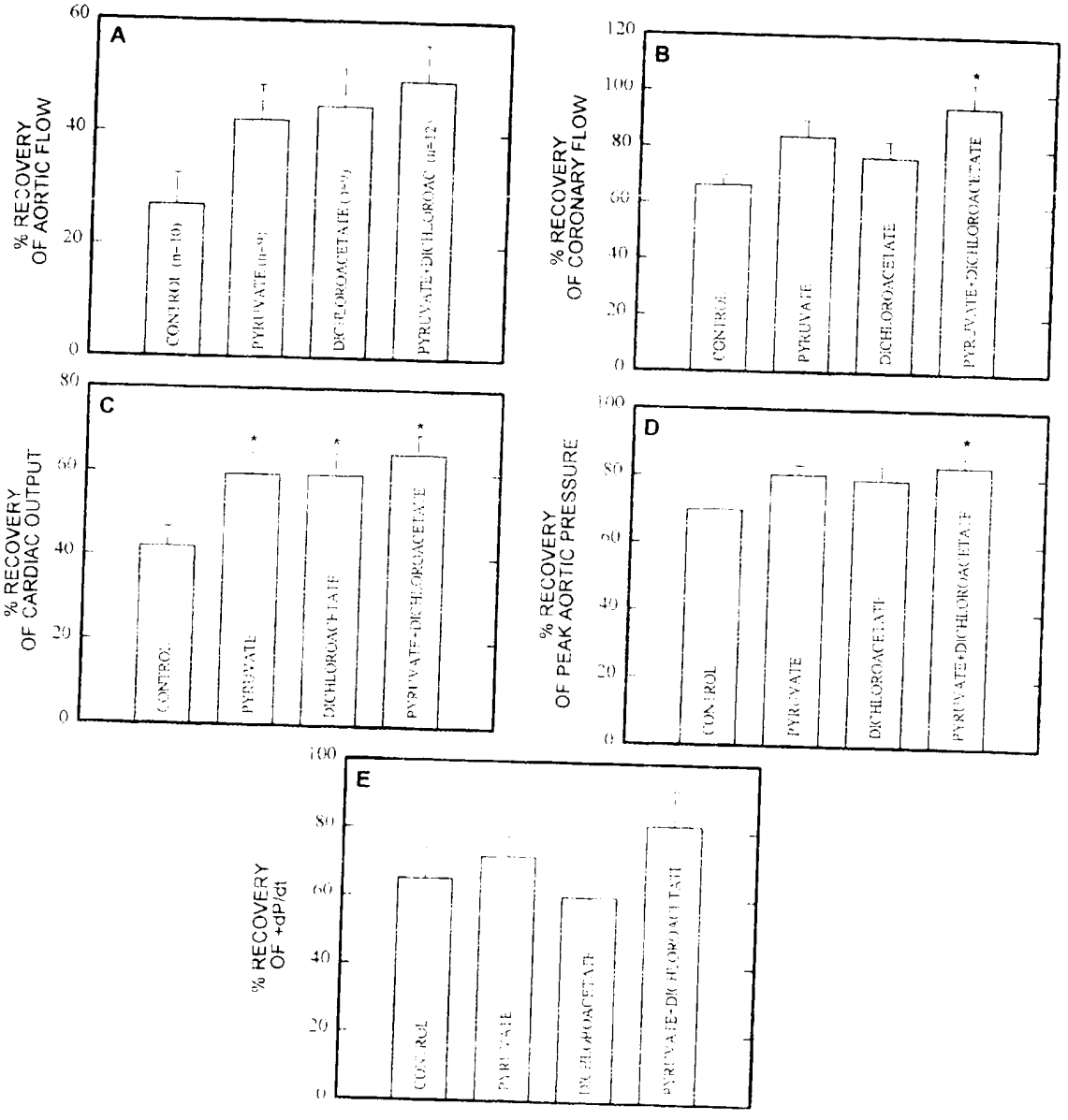
the experiments performed were excluded from data analysis. Five additional hearts were freeze-clamped after 10 min of Langendorff perfusion without ischaemia to determine the initial metabolite contents.

2.3.2. Langendorff perfusion for NMR spectroscopy

In NMR experiments (Fig. 1B), the protocol was similar to perfusion conditions in working mode, except that Langendorff perfusion was used throughout, the preischaemic perfusion time was 40 min due to the requirement of NMR spectrometer calibration, and only two experimental groups, control (C) and infused with pyruvate/dichloroacetate during first 15 min of reperfusion (PD), were used, with $n = 6$ in each group. No exclusion criteria were used for these experiments. NMR spectroscopic analysis was performed as described below.

2.4. ^{31}P NMR spectroscopy

Changes in myocardial ATP, PCr and Pi were followed using ^{31}P NMR (Bruker AMX-400 wide bore vertical system, ^{31}P frequency, 161.9 MHz) as described previously [12]. Fully relaxed spectra were acquired at 20 min of normoxic perfusion (36 scans, 90° angle and 15 s interpulse delay). Subsequently, saturated spectra (12 or 280 scans, 60°



angle and 2 s interpulse delay) were collected throughout the experiment. An initial ATP concentration of 23 μmol/g dry wt, as measured by HPLC, was used for calibration of the NMR data. For calculation of high-energy phosphate levels in saturated spectra, factors obtained from repeated fully relaxed and saturated spectra acquired during baseline conditions were used. Correction of the saturation factor for calculations at 8°C was obtained after repeated acquisitions at 37 and 8°C in a solution of ATP, PCr and Pi with intracellular concentrations of inorganic ions.

2.5. Metabolic determinations

Purine concentrations in the coronary effluent collected during the first 15 min of reperfusion and nucleotide content in the extracts of hearts freeze-clamped at the end of experiments were analyzed by HPLC. Coronary effluent samples were directly injected into the chromatograph. Freeze-clamped hearts were first freeze-dried, and subsequently, about 20 mg of freeze-dried tissue was homogenized with 0.5 ml of 0.4 M perchloric acid. After centrifugation to remove protein precipitates, the supernatant was neutralized with 2 M potassium hydroxide. After a second centrifugation to remove potassium perchlorate, samples were injected into the chromatograph. Details of the reversed-phase chromatographic procedure have been described previously [13]. Troponin I concentration in the coronary effluent was evaluated using radioimmunoassay in a pooled coronary effluent collected over the 15 min reperfusion period.

2.6. Statistical analysis

All results are expressed as means ± SEM. One-way analysis of variance (ANOVA) was used for comparison of the functional recovery of different groups, while repeated measures ANOVA was used to analyze coronary flow changes. For data not fulfilling a normal distribution and equality of variance criteria, ANOVA on ranks was used. ANOVA was followed by the Student Newman Keuls test to identify individual differences. Differences were considered significant with a value of $P < 0.05$.

3. Results

3.1. Functional recovery after cardioplegic arrest

Fig. 2 presents the percentage recovery of coronary flow and mechanical function after cardioplegic arrest. Coronary flow recovery was significantly improved in hearts treated with pyruvate/dichloroacetate (PD; Fig. 2B). The cardiac output (Fig. 2C) was significantly improved by the administration of pyruvate (P) or dichloroacetate (D), but the best recovery was observed after the administration of both compounds together (PD). The baseline absolute values were: 23.2 ± 1.6, 25.1 ± 2.3, 22.9 ± 1.1 and 21.1 ± 2.0 ml/min for coronary flow; 35.9 ± 2.4, 33.2 ± 2.0, 28.6 ± 2.4 and 33.3 ± 1.6 ml/min for aortic flow; 59.1 ± 2.6, 56.1 ± 2.6, 53.6 ± 3.2 and 53.6 ± 2.9 ml/min for cardiac output in C, P, D and PD groups, respectively. The PAP values (Fig. 2D) also demonstrated a significant improvement in PD, while the values in P or D were not significantly different from C. Baseline absolute values of PAP were 184.2 ± 9.2, 170.0 ± 11.2, 175.1 ± 12.8 and 172.9 ± 8.0 mmHg in C, P, D and PD, respectively. There were no significant differences in dP/dt values between the groups (Fig. 2C). The coronary flow evaluated during the Langendorff perfusion phase before and after ischaemia is presented in Table 1. There was a trend for increase in coronary flow in all treated groups, especially in the early phase of reperfusion.

3.2. Metabolite contents in the heart, purine catabolite and troponin I release

There were no significant differences in ATP or phosphocreatine content at the end of the experiments performed according to protocol A (working mode), as shown in Table 2. No differences were observed in the concentrations

Table 1
Coronary flow measured before ischaemia and during reperfusion in hearts treated with pyruvate, dichloroacetate or with both compounds in working mode experiments[a]

		Preischaemic (ml/min)	Reperfusion			
			0–0.5 min (% of initial)	0.5–2.5 min (% of initial)	2.5–5 min (% of initial)	5–15 min (% of initial)
Control	Mean	15.3	94	84	84	99
	SEM	1.4	13	10	11	12
Dichloroacetate	Mean	16.3	114	93	96	99
	SEM	0.8	12	13	10	7
Pyruvate	Mean	13.6	116	106	110	112
	SEM	0.6	11	7	7	8
[illegible]	Mean	14.8	118	103	100	100

Table 2
Concentrations[a] of nucleotides, phosphocreatine and creatine at the end of experiment in hearts treated with pyruvate, dichloroacetate or both in working mode experiments[b]

		Phosphocreatine	Creatine	GTP	GDP	ATP	ADP	AMP	NAD	ADPR	NADP	Adenosine
Control	Mean	32.6	25.7	0.86	0.20	13.09	3.59	0.68	4.13	0.31	0.26	0.029
	SEM	2.2	1.4	0.03	0.02	0.44	0.17	0.23	0.17	0.06	0.02	0.010
Dichloroacetate	Mean	34.4	29.1	0.74	0.20	13.50	3.84	0.93	4.14	0.39	0.27	0.044
	SEM	5.4	3.1	0.14	0.02	0.68	0.22	0.49	0.22	0.14	0.02	0.029
Pyruvate	Mean	40.0	24.3	0.80	0.19	12.87	3.32	0.47	4.29	0.35	0.25	0.031
	SEM	2.2	1.3	0.03	0.01	0.73	0.07	0.08	0.13	0.03	0.01	0.013
Pyruvate + dichloroacetate	Mean	32.6	32.0	0.88	0.18	14.21	4.05	0.63	4.16	0.32	0.26	0.022
	SEM	3.6	3.3	0.06	0.01	1.44	0.29	0.09	0.13	0.06	0.01	0.003

[a] Concentrations are given in μmol/g dry weight
[b] Values are means ± SEM; $n = 9$–12.
[c] GTP, guanosine triphosphate; GDP, guanosine diphosphate; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; NAD, nicotinamide adenine dinucleotide; ADPR, adenosine diphosphoribose; NADP, nicotinamide adenine dinucleotide phosphate

of any other metabolites measured. The total purine release over the 15 min of reperfusion was also not different among the groups; the total amounts were 1878 ± 122, 1729 ± 135, 1776 ± 122 and 1716 ± 115 nmols in C, P, D and PD groups, respectively. Troponin I release from the heart into the coronary effluent measured over 15 min of reperfusion was not different among the groups. The total amounts released in the 15 min of reperfusion were 61.1 ± 20.8, 71.4 ± 21.8, 75.7 ± 11.7 and 47.9 ± 19.1 ng of troponin I in C, P, D, and PD, respectively.

3.3. NMR studies of high-energy phosphate concentration changes

Fig. 3 shows changes in the phosphocreatine and ATP concentrations before, during and after cardioplegic arrest and ischaemia in an experiment performed according to protocol B. After an initial increase during cardioplegic infusion, the phosphocreatine concentration decreased gradually to the zero level after 3 h of ischaemia. During reperfusion, phosphocreatine was restored rapidly in the PD group, while some delay was observed in control hearts (Fig. 3C). The final concentrations of phosphocreatine at the end of the experiment were similar in both groups. There were no differences in ATP concentration changes (Fig. 3B) or inorganic phosphate between the groups (not shown). It was not possible to quantitate ATP or inorganic phosphate signals during the early phase of reperfusion.

4. Discussion

The major finding of this study is the demonstration that the addition of pyruvate or dichloroacetate at the time of reperfusion improves the mechanical function of the heart subjected to prolonged hypothermic cardioplegic arrest and that the mechanism of this effect may involve an accelerated [illegible]

[illegible]

administration were demonstrated previously after normothermic ischaemia and in an experimental infarction model [6,14,15], but, to our knowledge, only in two studies was the effect of dichloroacetate or pyruvate evaluated in an experiment which included infusions of cardioplegic solution [16,17]. However, only mild (34°C) hypothermia was maintained during the ischaemic phase in one [16], while the second focused on pretreatment with pyruvate before cardioplegic arrest [17]. Our study extends these findings, showing that in a clinically relevant model of myocardial ischaemia, pyruvate and dichloroacetate also exert beneficial effects if applied only during reperfusion.

We have previously demonstrated severe abnormalities of pyruvate metabolism in the donor heart after transplantation. Initially, apparent uptake of pyruvate in the heart was observed, while later, pyruvate was released [2]. This was in line with the concept that due to a high NADH/NAD ratio at the start of reperfusion, pyruvate was extracted and converted into the lactate in the lactate dehydrogenase reaction, while in the following phase after normalization of the NADH/NAD ratio, pyruvate was released, most likely due to inhibition of the pyruvate dehydrogenase complex. The present study was thus a logical attempt to propose the means of overcoming these metabolic abnormalities and to correlate it with functional effects. The increase of pyruvate concentration during reperfusion applied here was used not only to increase the metabolic flux through the pyruvate dehydrogenase reaction, but also to facilitate normalization of the NADH/NAD ratio in the heart and to exert its free radical scavenging effects [18–20]. Facilitated normalization of NADH/NAD ratio would prevent inhibition of the glycolytic pathway by NADH accumulation, allowing partial compensation for the deficit of oxidative high-energy phosphate synthesis observed during very early reperfusion [2,21].

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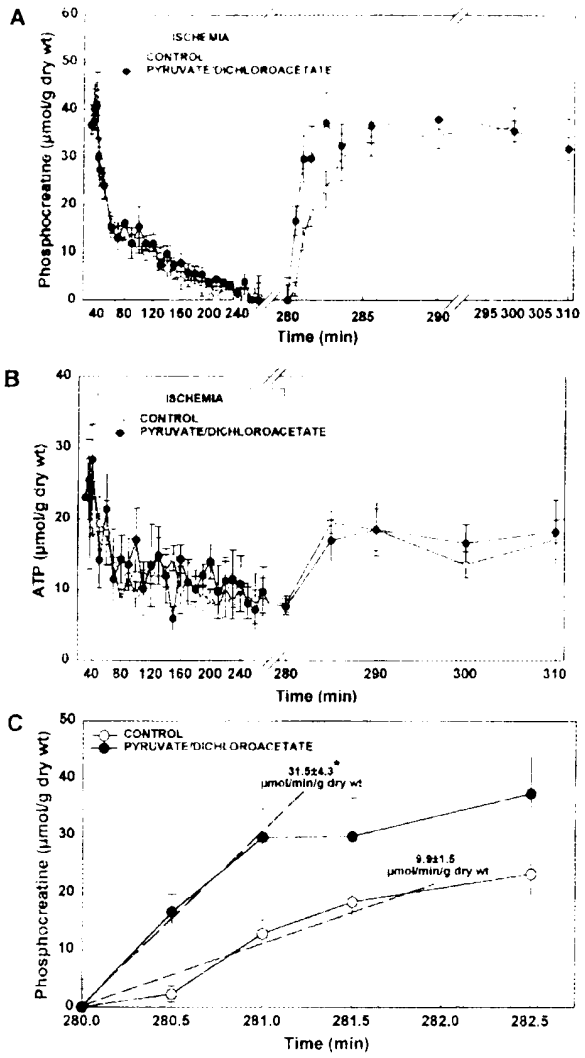

Fig. 3. (A) Phosphocreatine; and (B), ATP concentration before, during and after cardioplegic arrest in controls and hearts reperfused with pyruvate and dichloroacetate. (C) The recovery of phosphocreatine during initial phase of reperfusion together with calculated rate is presented. Hearts were arrested at 40 min of experiment and reperfused at 280 min. Values are expressed as means ± SEM; $n = 6$; *$P < 0.001$ vs. control.

observed. This may play a crucial role in the protective effect of pyruvate/dichloroacetate. Early reperfusion is a very critical time when the majority of oxygen radical related damage and calcium overload occurs. It is a time when ionic balance is restored in the cell. All these processes are energy dependent and when increased energy demand is not matched with adequate supply, further myocardial damage occurs. Therefore, rapid restoration of high-energy phosphate synthetic capacity is important during this period. We have previously shown that a decreased rate of recovery of the energy equilibrium may be the mechanism for deterioration in functional recovery

[illegible] suggested that delayed restoration of the energy equili-

brium could be a problem specific to recovery after cardioplegic arrest and hypothermic ischaemia, because after normothermic ischaemia, phosphocreatine concentration recovery is much faster. Rapid restoration of the energy equilibrium could thus be an important target for pharmacological intervention after hypothermic ischaemia.

The ATP concentration at the end of the experiment was similar in all of the groups, as was the phosphocreatine content, both in working mode and NMR experiments. This could be due to the vast majority of nucleotide breakdown occurring during the ischaemic phase while metabolic interventions were undertaken during reperfusion. In line with the measurements of ATP in the heart, the total purine catabolite release was similar in all of the groups. A trend for higher values of coronary flow during reperfusion in P, D and PD groups was observed (Table 1) and recovery of the coronary flow measured during assessment of function was significantly improved in the PD group (Fig. 2B). Dissociation of the beneficial functional effect from the end-point content of intracellular high-energy phosphate metabolites is in agreement with the previous studies on application of dichloroacetate during reperfusion [6]. This also confirms that it is important not only to follow concentrations at the end, but also changes throughout the experiment and the rate of restoration of various metabolic indices.

Activation of the pyruvate dehydrogenase flux seems to be the most likely factor responsible for the beneficial functional effect of pyruvate and dichloroacetate administration and for accelerated restoration of the energy equilibrium. It is known that in the crystalloid perfused heart replacement of glucose by pyruvate as a metabolic substrate results in the elevation of phosphocreatine concentration [22]. Similar effects may occur during early reperfusion with increased pyruvate concentration and its accelerated metabolism. Combined application of pyruvate and dichloroacetate seems to have additive beneficial effects in our experiments, although this is difficult to explain since pyruvate dehydrogenase complex inactivation was already prevented by 2 mM pyruvate alone [9]. Pyruvate alone has also been found to be superior compared with dichloroacetate when supplied during reperfusion after low flow ischaemia [23]. However, all studies relevant to this problem were performed at normothermia, and it is not known what the effect of hypothermic ischaemia on pyruvate dehydrogenase complex activity is. Our clinical data obtained after heart transplantation [2], showing a substantial release of pyruvate even after 45 min of reperfusion, suggest that this inhibition could be substantial and lasts much longer than after normothermic ischaemia [6]. Consequently, the administration of pyruvate alone may not be sufficient to fully reactivate the pyruvate dehydrogenase complex in this situation. Clarification of whether a further increase of pyruvate concentration would provide similar benefits to the combined application of pyruvate and dichloroacetate

[illegible] application between

effects and troponin I release from the heart. Troponin I could be released from the cell due to structural changes of the cell membrane. These changes could be predominantly attributed to the ischaemic phase, but a substantial proportion may occur during reperfusion as a consequence of free radical attack. It appears therefore that pyruvate/dichloroacetate addition does not prevent reperfusion induced changes of the membrane structure despite the free radical scavenging properties of pyruvate [19]. Pyruvate has been identified as a potent anti-oxidant in various models of myocardial ischaemia, which has been mainly related to stabilization of the redox potential and inhibition of NADH oxidase [24]. The anti-oxidant properties of pyruvate were found to be responsible for a protective effect if pyruvate was administered before ischaemia or in cardioplegic solution, so perhaps, some time delay may have contributed to the lack of clear effect on this parameter [17]. On the other hand, vascular function seems to be better preserved in hearts treated with pyruvate/dichloroacetate as indicated by a better recovery of coronary flow. Since oxidative stress at the time of reperfusion rather then metabolic injury is considered to be a major factor affecting vascular function in the postischaemic heart, this improved recovery may be attributed to the anti-oxidant effects of pyruvate.

In the present experiments, we added lactate and pyruvate to our standard perfusion buffer at concentrations close to values measured at the time of reperfusion during heart transplantation in humans [2]. The concentration of lactate was approximately 3 mM, while that of pyruvate was 0.1 mM. This modification was important since a high lactate concentration at the time of reperfusion may modify many effects of pyruvate or dichloroacetate in the heart. Furthermore, it is known that this elevated lactate concentration may exert a deleterious effect on the recovery of the heart [18] and pyruvate administration may protect against it.

Despite the benefits of the intervention proposed in our study, caution is necessary with the potential application of dichloroacetate in humans. This compound has been shown to exert teratogenic and carcinogenic properties in animal experimental studies, as well as other toxic effects [25]. Further studies are needed to determine whether these toxic effects are directly related to dichloroacetate or potential contaminants of early preparations. Dichloroacetate has been used in humans predominantly for the treatment of hyperlactacidaemia or congestive heart failure [26,27] with no apparent deleterious effects. Since pyruvate is a natural compound existing in body fluids it appears to be much safer for clinical applications. As an oral drug, it is used as a metabolic supplement in sports medicine and in the treatment of overweight patients [28]. It was also successfully used in humans for the treatment of heart failure via intracoronary infusion [29].

In conclusion, we have demonstrated that the addition of pyruvate and/or dichloroacetate during reperfusion exerts beneficial effects on myocardial function during clinical transplantation. The key process relating to the mechanisms of this enhanced functional recovery could be an accelerated restoration of the energy equilibrium during the early reperfusion period.

Acknowledgements

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